ELECTRON MICROSCOPIC STUDY ON THE METABOLISM OF INTRAVENOUSLY INFUSED FAT EMULSION

AUTHOR(S):

NAKAMURA, MASANORI

CITATION:

NAKAMURA, MASANORI. ELECTRON MICROSCOPIC STUDY ON THE METABOLISM OF INTRAVENOUSLY INFUSED FAT EMULSION. 日本外科宝函 1960, 29(3): 699-724

ISSUE DATE:

1960-05-01

URL:

http://hdl.handle.net/2433/207118

原　　著

ELECTRON MICROSCOPIC STUDY ON THE METABOLISM OF INTRAVENOUSLY INFUSED FAT EMULSION

by

MASANORI NAKAMURA

From the 2nd Surgical Division, Kyoto University Medical School
(Director : Prof. Dr. YASUMASA AOYAGI)
Received for publication March 23, 1960

I. INTRODUCTION

Investigation of the intermediate metabolic processes of fat are now being actively carried on in various fields. These processes, however, are not yet sufficiently clarified, especially when compared with protein and carbohydrate metabolism. It is no exaggeration to say that we are still quite in the dark even about the disposal of the chylomicra which are absorbed through the thoracic duct from the intestinal mucosa.

For many years our laboratory, paying attention to the nutritional value of fat, has tried not only to emphasize the necessity of sufficient pre- and postoperative supply of fat, but also to manufacture a fat emulsion which can be safely employed for intravenous injection[16]. At the same time investigations have been carried out of the metabolic processes responsible for the oxidation and utilization of intravenously administered fat emulsion[2)11)19)31)53)]. Our laboratory, with the use of a special technique, has succeeded in reducing triglyceride to fine droplets of nearly the same size as the physiological chylomicra which are absorbed from the intestinal mucosa after oral intake of fat. These fine droplets of triglyceride, when intravenously injected, are taken in by reticuloendothelial cells, and in these cells changed into phospholipid through the primary metabolic processes[2)19)53)]. These metabolites again go over into the blood stream in the form of lipoprotein, and are carried to the parenchymal organs, and there for the first time undergo a chiefly oxidative secondary process with the end products carbon dioxide and water[12)15)]. It has also been ascertained that a part of the injected fat may be stored, temporarily, for reserve energy[31)].

From a purely biochemical standpoint[1)28)], however, it has been asserted that chylomicra in the blood are further reduced by the lipoprotein-lipasic action of a clearing factor to particles small enough to penetrate capillary walls and cell membranes, and go directly into organ tissues in the form of glyceride. Thus even concerning the disposal of chylomicra there is as yet no definite agreement.

The present investigation was done by electron microscope to check our view

about the disposal of chylomicra. Special attention was directed to the following points:

a) Through what mechanism are the chylomicra in the blood stream taken into reticuloendothelial cells?

b) What changes does glyceride undergo in these cells?

c) How do the organellas of these cells behave during the metabolic processes?

In the present investigation the liver of the cat was chosen as experimental material: the liver possesses not only sinusoidal endothelial cells which participate in the primary metabolic process of glyceride, but also hepatic parenchymal cells, which are involved in the secondary process. Moreover, in hepatic sinusoidal endothelial cells there are no granules which might be mistaken for the injected fat droplets because of similarity of size and electron density[14].

II. MATERIALS AND METHODS

Cats weighing 2.5～3.5 kg in a postabsorptive state were used as experimental animals. Starved animals were chosen in order to keep the influences of diet to a minimum[2]. A 20% sesame oil emulsion was given intravenously to the experimental animals in 2.5 cc quantities per kg body weight. The quantity of fat thus administered amounted to 0.5g per kg body weight. After being diluted 1 : 3 with an isotonic saline solution the emulsion was slowly injected into the femoral vein taking more than 5 minutes. These animals were laparotomized under no anesthesia, immediately after injection, and 5, 10, 20 and 45 minutes later, and one, one and a half, 2, 3, 4, 5 and 6 hours later. And then specimens of hepatic tissue of a certain size were excised from a certain section of the right lobe of the liver, immediately cut to pieces about $1mm^3$ in size, put in a M/25 isotonic sucrose solution containing 1% osmic acid[3)52)] which was maintained at pH 7.2 with a veronal buffer solution[41], and fixed for 2 hours in a refrigerator. For dehydration of the tissue a series of alcohol solutions was employed. (The tissue was put for 10 minutes each in 30%, 40%, 50%, 75%, 90% and 95% alcohol solutions, and twice for 30 minutes in 100% alcohol.) The tissue was embedded in a mixed solution of n-buthyl- and methyl-methacrilate (6 : 4), and kept in a refrigerator for 24 hours. The solution was then tubed into No. 0 capsules, embedded, and heated at 55°C in an incubator for polimerization.

Specimens were similarly prepared from starved controls, and also from mice which had received cod liver oil parenterally. The hepatic sinusoidal endothelial cells of the experimental animals feeding on fat were also examined.

A NIPPON ultramicrotome was used in preparing ultrathin section, and observation was done with an AKASHI Tronscope TRS-50E-type. Photographs were taken under 4000～7000 magnifications.

III. OBSERVATIONS

1) Normal Structure of the Hepatic Sinusoidal Endothelial Cell

Sinusoids run parallel to each other along both sides of the liver cell trabecula

(Fig. 1). In places hepatic sinusoidal endothelial cells protrude into the sinusoidal lumen, sometimes filling the entire space. Immature endothelial cells, poor in cytoplasm, lie on the wall surface (Plate 1, 2 and 4). Endothelial cells are extremely irregular in shape with many pseudopodlike cytoplasmic protrusions and infoldings (Plate 2 and 4). These cells each possess one nucleus usually kidney-shaped. As above-mentioned, cytoplasm, in comparison with the nucleus, is scanty in immature cells, but rather abundant in the mature. On the whole, there are few fine granules in the cytoplasm, which therefore looks rather pellucid. Well-developed endoplasmic reticula are clearly recognizable. The smooth-surfaced ones are present in ample numbers especially in the peripheral part of the cell, while the rough-surfaced ones are noted in small numbers only in the central part of the cell, i. e. around the nucleus. The smooth-surfaced endoplasmic reticulum is clearly connected with a cytoplasmic infolding, and sometimes its inner space is greatly swollen, and forms a vacuole. PALADE's so-called RNA granules about 200 Å in diameter are seen sticking to the rough-surfaced endoplasmic reticulum (Plate 3). The mitochondrium of the endothelial cell is less than two-thirds the size of that of the hepatic parenchymal cell, but its mitochondrial membrane is composed of double membrane, and cristae mitochondriales are clearly recognizable, as in the case of all other cells of the body (Plate 3). The GOLGI complex is present in the nuclear depression, and such structures as GOLGI vesicles and lamellae are also observable, but they are poorly developed and vestigial.

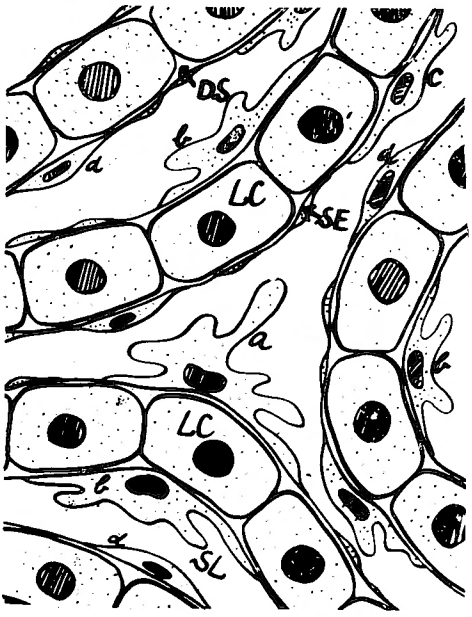


Fig. 1.
Diagramatical Illustration of Liver Structure. LC: Liver Cell, SL: Sinusoidal Lumen, SE: Sinusoidal Endothelium, DS: DISSÉ's Space, a, b, c, d: Sinusoidal Endothelial Cells.

The above is an outline of the fundamental structure of the normal sinusoidal endothelial cell. Besides the above findings, mildly-osmiophilic, irregularly-shaped granules are sometimes noted, but it seems hasty to conclude that these granules are fat droplets (Plate 4). Definitely osmiophilic granules such as those always present in neutrophils, eosinophils and basophils are not detected. Phagocytized erythrocytes, too, are often found in sinusoidal endothelial cells. Erythrocytes which have just been phagocytized uniformly show a high electron density, and look structureless, but with the lapse of time ferritin is freed and can be recognized as so-called ferritin granules about 45 Å in size[20)30)59)]. Sometimes these granules are seen widely and abundantly distributed in every part of the cell (Plate 8 and

18).

Sinusoidal endothelial cells, the cytoplasm of which is stretched thin at both ends, constitute a sinusoidal endothelium. This endothelium, having many pores, presents a meshwork-like appearance (Plate 1 and 2); and, unlike other capillary endothelia[20)36)51)], possesses no basement membrane, and directly touches the microvilli of hepatic cells. Optic microscopically speaking, the so-called Dissé's space is situated between it and the microvilli (Plate 1, 2, 3 and 5). Accordingly, all the blood components, except corporeal elements, freely enter Dissé's space; in other words, hepatic cells are in direct contact with the blood which streams in through the pores of the sinusoidal endothelium. From this it may be said that the optic microscopic conception of Dissé's space as a pure lymph space does not correspond to reality. Collagen fibers are often noted to run in the boundary line between two hepatic cells (Plate 10). In hepatic parenchymal cells fat droplets decrease in number in a fasting state, but besides this characteristic findings, the findings obtained from observation of the fine structure of these cells were the same as have already been reported[7)9)13)49)].

2) Intake by Sinusoidal Endothelial Cells of Fat Globules from the Blood Stream, and Changes of the Globules in These Cells

Droplets of glyceride which are intravenously injected in the form of emulsion are observable in large number on the surface of sinusoidal endothelial cells immediately after injection, and some of the droplets are already taken into the cells (Plate 6). These droplets of glyceride are about 0.3μ in diameter, and characteristically uniform in size. This finding stands in striking contrast to the fact that fat droplets which appear in the lymph vessels in the submucous tissue of the small intestines after oral intake of fat are irregular in size, though they are all less than 0.5μ in diameter (Plate 6, 7, 8 and 19).

With the lapse of 5, 10 and more minutes after injection of fat emulsion, droplets of glyceride taken into endothelial cells gradually increase in number. The cells take in droplets either one by one or several at a time. When several droplets are taken in a time, these droplets gradually coalesce into a larger droplet (Plate 7, 8 and 9). Detailed observation of the manner in which endothelial cells take in droplets of glyceride discloses the following: The cell membrane of the endothelial cell first catches droplets of glyceride. As shown in Fig. 2, whether one droplet or several are caught, the part of the cell membrane which touches droplets is gradually depressed till it infolds and entirely encircles the caught drop-

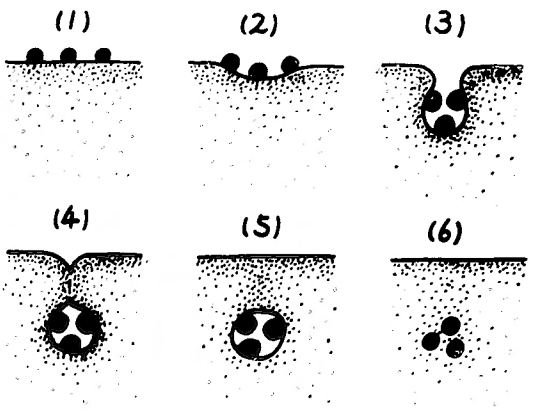


Fig. 2.
Diagram Showing the Concept of Membrane Vesiculation as Mechanism for Active Transport (modified from Bennett, H. S.).

lets. On this occasion the caught droplets are encircled as a unit even when they are several in number. And then the mouth of the infolded part closes, and thus a sort of phagovacuole, so to speak, comes into existence. Such being the case, phagovacuoles are of various sizes; the more droplets of glyceride they contain, the larger they are. When several droplets are caught, they coalesce into one larger droplet in the phagovacuole, but of course there are times when one droplet is caught singly; and frequently several droplets are taken in one by one in succession, and form a row (Plate 9). Phagovacuoles formed in this way are then completely cut off from their connection with the cell surface. In this finished state they are seen lying scattered in endothelial cells. In short, droplets of glyceride taken into the endothelial cell are encircled by the encircling membrane which has originated from the cell membrane (Plate 7, 8 and 9), and droplets in the phagovacuole finally unite with one another into one unit, and these units are seen here and there in the cell; they are not necessarilly collected in the place where the GOLGI complex is situated.

As seen in the above description, droplets of glyceride always go into endothelial cells and there gradually undergo metabolic changes; they are never observed going directly into DISSÉ's space, and entering hepatic parenchymal cells from there. The injected droplets of glyceride completely disappear from the blood stream 30~45 minutes after injection of fat emulsion, putting an end to the artificially-produced lipemia[2)16)].

Droplets of glyceride taken into the endothelial cell coalesce into one larger droplet in the phagovacuole, as mentioned above, but the shape of this larger drop is gradually distorted with the breaking-up of the encircling membrane (Plate 10). As the nearly complete disappearance of the membrane is noted two hours later, the droplet may naturally be assumed to have freed itself into the cytoplasm of the endothelial cell by this period (Plate 14). Starting about one hour after injection of fat emulsion the mitochondria in the endothelial cell gradually increase in number, and when the encircling membrane begins to lose its definite structure, come to gather around fat droplets, making contact with them. When the outer membrane of the mitochondrium comes in contact with the fat droplet, it becomes indistinct, and the crista mitochondrialis touching the droplet is characteristically placed at right angles to the latter. Thus morphologically the fat droplet steadily becomes irregular in shape, and indistinct in outline, and finally disappears; that is, three hours after injection of fat emulsion fat droplets are hardly detectable in the endothelial cell, as is the case in a fasting state.

With the progress of metabolism of the injected fat in the endothelial cell, namely, about one hour after injection of fat emulsion, particles of fat about 300~600 Å in size begin steadily to increase in amount in DISSÉ's space; and when the metabolic activity reaches its height in the endothelial cell, these particles in DISSÉ's space also become most copious (Plate 10 and 11). In the present investigation, however, it was impossible to give morphological evidence that these particles of fat in DISSÉ's space had come directly from endothelial cells. Two or three hours

after injection of the emulsion, when the particles of fat appear most abundantly in Disse's space, fat droplets of various sizes make their appearance in hepatic parenchymal cells. It is reasonable to consider, in view of the above findings, that the injected fat, instead of entering directly into the hepatic parenchymal cells, first undergoes metabolic changes in hepatic sinusoidal endothelial and other reticuloendothelial cells, and then goes out into the blood stream as extremely fine particles of fat; and that these fine particles enter the hepatic parenchymal cells through Disse's space. In the parenchymal cells these particles again coalesce into fat droplets of various sizes. Actually a picture of small droplets adjoining big ones is often observable, which probably suggests the process of recoalescence (Plate 16). Five or six hours after injection of fat emulsion fat droplets vanish from hepatic sinusoidal endothelial cells (Plate 17 and 18).

In animals feeding on fat the increase in amount of fat droplets in hepatic sinusoidal cells is likewise cleary noted, though it is not so remarkable as in the above-mentioned case of intravenous injection of fat emulsion. From this it may be inferred that fat orally administered undergoes nearly the same metabolic processes as that intravenously injected.

IV. SUMMARY AND DISCUSSION

Recent electron microscopic studies[47)48)] have revealed that orally administered fat is absorbed through the intestinal mucosa into the lymph vessels in the form of chylomicra; and that these chylomicra enter the blood stream through the thoracic duct. And it has been considered by some investigators that these chylomicra measuring less than 0.5μ in diameter stay for a while in the blood stream as a serum suspension, but are gradually reduced to extremely small particles, and thus in the form of glyceride entre directly into the tissue of various organs, penetrating the capillary wall and the cell membrane; and that when the particles have entered the organs, the opacity of the blood is cleared. N. K. Freeman and others called the factor that participated in the clearing process of blood a clearing factor, and stated that heparin greatly promoted this process[1)].

The results of the histochemical investigations conducted in our laboratory after intravenous injection of fat emulsion or oral administration of fat[2)19)53)], however, have shown that reticuloendothelial cells play a big role in the primary metabolic process of chylomicra[38)56)]: these cells contribute to the clearing of lipemia by taking droplets of glyceride into themselves, and subjecting them to the primary metabolic process; in these cells glyceride gradually changes into phospholipid, and after being reduced to particles, is again freed into the blood stream, and then absorbed into parenchymal organs in the form of lipoprotein to undergo the secondary metabolic process. Such being the results obtained by our laboratory, the present author finds it impossible to agree with the above-mentioned view that chylomicra are already reduced to smaller particles in the blood stream, and in the form of glyceride enter directly into parenchymal organs to be oxidized. In order to solve this problem the present author, after previously reducing glyceride to

particles, intravenously injected the emulsion into experimental animals, and investigated the metabolism of glyceride from a morphological standpoint, using an electron microscope because of its great resolving power.

The technique of preparing ultrathin section using the osmic acid fixation method developed by G. E. PALADE, F. S. SJÖSTRAND and others has brought about remarkable progress in electron microscopic studies of cellular ultrastructure, and it is now common knowledge that this progress has enabled us to ascertain not only the morphology, but even the functions of intracellular organellas[3)41)52)]. In addition it has become possible through ultracentrifugation to separate and refine the tissue homogenate into different intracellar organella, and the function of individual organellas, too, have become enzymologically ascertainable. Under these circumstances it is no exaggeration to say that present-day cytology has reached the stage of intracellular organella investigation.

In the present investigation the metabolic processes of fat globules intravenously injected in the form of emulsified glyceride were studied with an electron microscope, choosing the liver as the object of observation. Glyceride intravenously injected in the form of droplets less than 0.5μ in diameter is actively absorbed by hepatic sinusoidal endothelial cells, as shown in Plate 6, 7, 8 and 9. But on this occasion droplets of glyceride in the blood stream in the sinusoid are never observed to occlude a sinusoidal lumen by coalescing with one another into a large droplet. It has thus been ascertained that the intravenous injection of the fat emulsion prepared by our laboratory has no danger whatever of causing a sinusoidal occlusion. Moreover, these droplets of glyceride in the sinusoid are all less than 0.3μ in diameter, and their individual differences in size are not noticeable as in the case of the chylomicra which appear in the lymph vessels of the submucous tissue of the small intestines after oral administration of fat. In view of the fact that droplets of glyceride in our fat emulsion are all less than 0.3μ in diameter, while many of the fat globules which appear in the lymph vessels after oral administration of fat are as large as 0.5μ, our fat emulsion may safely be employed for intravenous injection, and the intravenous injection of glyceride in the form of emulsified fine droplets may be regarded as an adequate method of supplying fat nutrition. It should be particularly borne in mind that this emulsion which is a sort of very thick colloidal solution is to be diluted more than 1 : 3 with an isotonic saline or RINGER'S solution before intravenous injection, and to be injected very slowly, for colloidal shock is a inevitable accompaniment of the intravenous injection of colloids. If care is exercised in the way described above, our fat emulsion is an extremely safe agent which causes no side reactions[16)31)].

Many of the droplets of glyceride thus intravenously injected are noted on the surface of sinusoidal endothelial cells as early as 5 minutes after commencement of injection, and some of them are already taken into the cells. The cell membrane of these cells may be regarded as endowed with remarkable ability to catch the droplets of glyceride in the blood stream. Droplets of glyceride which touch the cell membrane are caught, and taken as a unit regardless of their number into the

phagovacuole originating from the cell membrane. These droplets caught as a unit unite with one another into one larger droplet in the phagovacuole, and these phagovacuoles are seen lying scattered in the cell.

In the present investigation the endothelial cell was not observed to catch droplets of glyceride with pseudopodlike protrusions, nor were the droplets seen to enter the open mouth of an endoplasmic reticulum on the cell surface, and thence proceed through the reticulum into the cell, as had been reported by H. UCHINO and others [42)50)55)]. R. J. BENNETT advanced the membrane flow theory concerning the mechanism of cellular absorption[5)]. According to this theory, as shown in Fig. 2, the part of the cell membrane which is touched by some absorbable substances is gradually depressed, and then the mouth of the infolded part closes, thereby forming a sort of vacuole. The substances thus caught go over into the cytoplasm when the vacuole disintegrates. In the present investigation nearly the same phencmena as the above were obserrved when the endothelial cell took in droplets of glyceride; that is, a depression occured in the part of the cell membrane which was touched by droplets of glyceride, and one or several droplets, as the case might be, were caught as a unit by the indented membrane, which after a while was completely disconnected from the surface membrane of the cell. In this way a sort of phagovacuole was formed. When several droplets of glyceride were simultaneously taken into the phagovacuole, they gradually coalesced into one larger droplet. Sometimes one droplet was caught singly.

After some time the wall of the phagovacuole, i. e., the encircling membrane begins to lose its definite structure, and finally completely vanishes. When this occurs, fat droplets are freed into the cytoplasm. It is stated elsewhere that the encircling membrane persists without disintegration for more than 24 hours when foreign bodies injurious to the living organism enter the hepatic sinusoidal endothelial cell instead of vanishing in so short a time as in the above case[14)].

About one hour after injection of fat emulsion mitochondria characteristically begin to increase in number in the endothelial cell which is engaged in the metabolism of fat droplets, and gradually come to gather around the ingested droplets, and make contact with them. The part of the mitochondrial outer membrane touching the droplet become very indistinct, and the cristae mitochondriales is placed at right angles to the droplet. These findings are in accord with the results of observation by phase-contrast microscope by J. J. BIESELE[54)] and others that mitochondria increase in number, and become more active with the augmentation of metabolic activity of the cell. Though the cytoplasm of the living cell is in a state of flux, these electron microscopic findings are believed to represent momentary phases of cellular activity; and so mitochondria and fat droplets may be assumed to be now touching and now parting from each other. Results of the present investigation leave no room for doubt that the primary metabolic process of the injected fat in the endothelial cell is conducted through the action of mitochondria.

J. D. LEVER[33)34)], in his electron microscopic observation of adipose tissue, found fat droplets in contact with mitochondria, and further G. E. PALADE[46)], T. YAMAMOTO

[60] and others observed in their investigations of hepatic and pancreatic cells in a fasting state that mitochondria were arranged around fat droplets, some the former actually being in contact with the latter, and thus established the functional connection between the two. They also reported that the outer membrane of the mitochondrium touching the fat droplet was very indistinct. In the present investigation, too, the mitochondria of the endothelial cell looked similar under the same circumstances. In short, the fat droplet taken into the endothelial cell seems to undergo a certain transformation under the influence of an enzymologic action. Moreover, the mode of contact between the mitochondrium and the fat droplet is really characteristic; with the start of disintegration of the encircling membrane, the crista of the mitochondrium touching the fat droplet is placed at right angles to the latter. As is commonly known, the mitochondrium contains various enzyme systems which participate in metabolism[4)58)], and needless to say, it also plays an important role in the oxidation and synthesis of fat. With the electron microscopic clarification of the fine structure of the mitochondrium, and with the recent progress of enzymologic studies many theories have been put forward concerning the arrangement of enzyme systems in the mitochondrium. Especially O. E. GREEN and his school's hypothesis that these enzyme systems are placed in the mitochondrial membrane and crista has been confirmed by the electron microscopic observations of G. E. PALADE[4)], M. C. WATSON and P. SIEKEVITZ[58)]. If this, GREEN's hypothesis is taken into consideration, the appearance of the mitochondrium observed in the present investigation when it was brought into functional contact with the fat droplet, namely, the disposition of the crista mitochondrialis at right angles to the latter, is well suited to the metabolism of fat.

As has been already histochemically and biochemically demonstrated by the colleagues of our laboratory[11)19)53)], it seems most reasonable to consider that the physiologic significance of functional contact between fat droplets and mitochondria lies in the transformation of glyceride into phospholipid through the action of mitochondria. Recently A. KORNBERG[29)], E. P. KENNEDY[21)~26)] and others, from an enzymologic standpoint, have studied minutely the process of formation of phospholipid from glyceride through the action of mitochondria, and in particular E. P. KENNEDY and his school have instituted an inquiry into the formative process of phospholipid, using the fraction of mitochondria separated by ultracentrifugation from the hepatic tissue homogenate, and succeeded in actually demonstrating the formation of phospholipid from glyceride through the action of mitochondria. Under these circumstances the participation of mitochondria in the primary metabolic process of glyceride taken into the endothelial cell shows the possibility of transformation in this cell of glyceride into phospholipid through the action of mitochondria. Moreover, if it is remembered that particles of fat in DISSÉ's space are about 300 Å in size, i. e. of the same size as the particles of fat which are used in the formation of alpha-lipoprotein; and that the majority of the latter particles are composed of phospholipid, the inference that the injected fat is transfered by the blood stream always through DISSÉ's space from sinusoidal endothelial cells into

hepatic parenchymal cells in the form of phospholipid, as particles of alpha-lipoprotein may be regarded as very pertinent.

V. CONCLUSIONS

In the present investigation triglyceride was made into an emulsion composed of fat globules less than 0.5μ in size, and this emulsion was intravenously injected into experimental animals. The metabolic processes of the injected glyceride were investigated by electron microscope, and the following conclusions were obtained.

1) Many of the droplets of glyceride intravenously injected are seen to have already been taken into hepatic sinusoidal endothelial cells 5 minutes after injection, and with the lapse of time the number of such ingested droplets increases. Droplets of glyceride in the sinusoid are all less than 0.3μ in diameter, that is, smaller than the large chylomicra which appear in the lymph vessels of the submucous tissue of the small intestines after oral administration of fat (The largest chylomicron measures 0.5μ in diameter.).

2) Droplets of glyceride are first caught by the encircling membrane which originates from the cell membrane. One or several droplets are caught as a unit, as the case may be, and when several droplets are taken in simultaneously, they coalesce with one another in the phagovacuole. When the encircling membrane finally disintegrates and vanishes, the droplets are freed into the cytoplasm of the cell.

3) When the encircling membrane begins to lose its definite structure, mitochondria increase in number, and come to gather around fat droplets, and some of them establish functional contact with the latter. On this occasion cristae mitochondriales are placed at right angles to fat droplets, and the part of the outer membrane of the mitochondrium touching the fat droplet becomes indistinct. Thus through the action of mitochondria fat droplets become irregular in shape, and blurred in outline, and finally completely disappear from the sinusoidal endothelial cell.

4) About this time many particles of about 300 Å in size make their appearance in Disse's space, and fat droplets increase in amount in hepatic parenchymal cells, too. These particles of fat are of nearly the same size as those particles of fat which are used in building up alpha-lipoprotein, the majority of which are made up of phospholipid.

5) The colleagues of our laboratory have previously histochemically and biochemically ascertained the metabolic processes in the living organism of glyceride intravenously injected. The above findings obtained by the present author are considered to verify the results of their investigations by electron microscope. In view of these experimental findings it seems most reasonable to think that the intravenously injected glycerides are first taken into hepatic sinusoidal endothelial and other reticuloendothelial cells, and after being changed into phospholipid in these cells through the action of mitochondria, transferred in the form of alpha-lipoprotein by the blood stream into the liver and other parenchymal organs where

these metabolites undergo further changes.

The author wishes to thank Dr. YORINORI HIKASA, the instructor of our clinic, for his many valuable suggestions and criticisms throughout the present investigation, and is also greaty indebted to Prof. Dr. MITSUGI NISHIURA for his kind guidance in electron microscopy.

REFERENCES

1) Anfinsen, C. B.: The role of heparin in lipoprotein metabolism. Science, **115**, 583, 1952.
2) Asada, S.: Histochemical studies on the intravenously infused fat emulsion. Arch. Jap. Chir., **22**, 77 et 217, 1953.
3) Bahr, G. E.: Osmium tetroxide and ruthenium tetroxide and their reactions with biochemically important substracts. Exp. Cell Res., **7**, 457, 1954.
4) Barnett, R. J. and Palade, G. E.: Histochemical demonstration of the sites of activity of dehydrogenase systems with the electron microscope. J. Biophysic. Biochem. Cytol., **3**, 577, 1957.
5) Bennett, H. S.: The concept of membrane flow and membrane vesiculation as mechanism for active transport and ion pumping. J. Biophysic. Biochem. Cytol., **2**, 99, 1956.
6) Brandt, P. W.: A study of the mechanism of pinocytosis. Exp. Cell Res., **15**, 300, 1958.
7) Brown, D. B. et al.: The electron microscopy of human liver. Gastroenterol., **32**, 103, 1957.
8) Dalton, A. J. and Felix, J. D.: A comparative study of the Golgi complex. J. Biophysic. Biochem. Cytol., **2**. suppl. 4, 79, 1956.
9) Fawcett, D. W.: Observations on the cytology and electron microscopy of hepatic cells. J. Nat. Canc. Inst., **15**, 1475, 1955.
10) Freeman, J. A.: The ultrastructure of the double membrane systems of mitochondria. J. Biophysic. Biochem. Cytol., **2**, 353, 1956.
11) Fujino, S.: Experimental studies on the fat matabolism by the use of radioactive phosphorus. Arch. Jap. Chir., **28**, 2668, 1959.
12) Fukata, T.: Experimental studies on fat metabolism in vivo with special reference to determination of alpha-ketoglutaric acid. Arch. Jap. Chir., **23**, 1631, 1959.
13) Furuta, Y.: Electron microscopic studies in the fine structure of liver. 1. Normal rat liver. Jap. Arch. Intern. Med., **3**, 535, 1957.
14) Hashimoto, M. et al.: Electron microscopic studies on the hepatic sinusoids of the mouse. Electronmicroscopy, **6**, 109, 1957.
15) Hashino, H.. Experimental studies on fat metabolism from the view-point of ketone body formation. Arch. Jap. Chir., **24**, 488, 1955.
16) Hikasa, Y.: Stuides on the fat metabolism by the use of fat emulsion. Saishin-igaku, **13**, 2278, 2586, 2954, 1958.
17) Holter, H.: Pinocytosis. Internat. Rev. Cytol., **8**, 481, 1959.
18) Ikeda, H.: Experimental studies on metabolism with a blocked reticuloendothelial system. Arch. Jap. Chir., **26**, 355, 1957.
19) Izukura, T.: Histochemical studies on intravenously administered fat emulsion. Arch. Jap. Chir., **26**, 215, 1957.
20) Karrer, H. E.: The ultrastructure of mouse lung: The alveolar macrophage. J. Biophysic. Biochem. Cytol., **4**, 693, 1958.
21) Kennedy, E. P.: Synthesis of phosphatides in isolated mitochondria. J. Biol. Chem., **204**, 399, 1953.
22) Kennedy, E. P.: Synthesis of phosphatides in isolated mitochondria. II. J. Biol. Chem., **209**, 525, 1954.
23) Kennedy, E. P. and Weiss, S. B.: Cytidine diphosphate choline; a new intermediate in lecithin biosynthesis. J. Am. Chem. Soc., **77**, 250, 1955.
24) Kennedy, E. P. and Weiss, S. B.: The function of cytidine coenzymes in the biosynthesis of phospholipids. J. Biol. Chem., **222**, 193, 1956.
25) Kennedy, E. P.: The synthesis of cytidine diphosphate choline, cytidine diphosphate etha-

nolamine and related compounds. J. Biol. Chem., **222**, 185, 1956.
26) Kennedy, E. P. et al.: The enzymatic synthesis of cytidine diphosphate choline. J. Biol. Chem., **227**, 951, 1957.
27) Klein, H. P. and Greenfield, S.: Effects of mitochondria on lipid synthesis in yeast homogenates. Exp. Cell Res., **17**, 185, 1959.
28) Korn, E. D.: Clearing facter, a heparin activated lipoprotein lipase. J. Biol. Chem., **215**, 15, 1955.
29) Kornberg, A. et al.: Enzymatic esterification of α-glycerophosphate by long chain fatty acids. J. Biol. Chem., **204**, 345, 1953.
30) Kuff, E. L. and Dalton, A. J.: Identification of molecular ferritin in homogenates and sections of rat liver. J. Ultrastr. Res., **1**, 62, 1957.
31) Kuyama, T.: Clinical studies on the nutritional effects of intravenous administration of fat emulsion. Arch. Jap. Chir., **27**, 64, 1958.
32) Lever, J. D.: Physiologically induced changes in adrenccortical mitochondria. J. Biophysic. Biochem. Cytol., **2**, 313, 1956.
33) Lever, J. D.: The fine structure of brown adipose tissue in the rat with observation on the cytological changes following starvation and adrenectomy. Anat. Rec., **123**, 361, 1957.
34) Lever, J. D. and Champell, J. B.: Mitochondria isolated from rat brown adipose tissue and liver. J. Biophysic. Biochem. Cytol., **4**, 287, 1958.
35) Low, F. N.: Mitochondrial structure. J. Biophysic. Biochem. Cytol., **2**, 337, 1956.
36) Maximow, A. A. and Bloom, W.: A textbook of histology. 17ed., 233, 1957.
37) Mende, T. J. and Chambers, E. L.: Studies on solute transfer in the vascular endothelium. J. Biophysic. Biochem. Cytol., **4**, 319, 1958.
38) Murray, R. G. and Freeman, S.: The morphological distribution of intravenously injected fatty chyle and artificial fat emulsion in rats and dogs. J. Lab. Clin. Med., **33**, 56, 1951.
39) Napolitano, L. and Fawcett, D.: The fine structure of brown adipose tissue in the newborn mouse and rat. J. Biophysic. Biochem. Cytol., **4**, 685, 1958.
40) Odor, D. L.: Uptake and transfer of particulate matter from the peritoneal cavity of the rat. J. Biophysic. Biochem. Cytol., **2**, 105, 1956.
41) Ogura, M.: Morphological research on the soluvent effect on the cytoplasmic granules with a electron microscope. J. Electronmicroscope, Japan, **5**, 198, 1957.
42) Palade, G. E.: Relations between the endoplasmic reticulum and the plasma membrane in macrophages. Anat. Rec., **121**, 445, 1955.
43) Palade, G. E.: A small particle conponent of the cytoplasm. J. Biophysic. Biochem. Cytol., **1**, 59, 1955.
44) Palade, G. E. and Siekevitz, P.: Pancreatic microsomes. J. Biophysic. Biochem. Cytol., **2**, 671, 1956.
45) Palade, G. E.: The endoplasmic reticulum. J. Biophysic. Biochem. Cytol., **2**, suppl. 4, 85, 1956.
46) Palade, G. E. and Schildowsky: Functional association of mitochondria and lipid inclusions (Abstract). Anat. Rec., **103**, 352, 1958.
47) Palay, S. L. and Karlin, L. J.: Absorption of fat by jejunal epithelium in the rat. Anat. Rec., **124**, 343, 1956.
48) Palay, S. L. and Karlin, L. J.: An electron microscopic study of the intestinal villus. II. The pathway of fat absorption. J. Biophysic. Biochem. Cytol., **5**, 373, 1959.
49) Parks, H. F.: The hepatic sinusoidal endothelial cell and its histological relationship. Electronmicroscopy (Proceed. Stockholm Confer.), 151, 1956.
50) Parks, H. F. and Chiquoine, A. D.: Observations on early stages of phagocytosis of colloidal particles by hepatic phagocytes of the mouse. Electronmicroscopy (Proceed. Stockholm Confer.), 154, 1956.
51) Peace, D. C.: Electron microscopy of the vascular bed of the kidney cortex. Anat. Rec., **121**, 701, 1955.
52) Porter, K. R. and Kallman, F.: The properties and effects of osmium tetroxide as a tissue fixative with special reference to its use for electron microscopy. Exp. Cell Res., **4**, 127, 1953.

53) Shirotani, H.: Histochemical studies on fat metabolism by intravenous administration of fatty chyle. Arch. Jap. Chir., **26**, 38, 1956.
54) Tobioka, M. and Biesele, J. J.: Mitochondria in living cells; an analysis of movements. J. Biophysic. Biochem. Cytol., 2, 319, 1956.
55) Uchino, F.: Electron microscopic studies on phagocytes with special reference to the relationship between phagocytosis and endoplasmic reticulum. Acta Haem. Jap., **20**, suppl., 63, 1957.
56) Waddel, W. R. et al.: Function of the reticuloendothelial system in removal of emulsified fat from blood. Amer. J. Physiol., **177**, 90, 1954.
57) Watanabe, Y.: Observations of white blood cell with electron microscope. J. Electronmicroscopy, **5**, 46, 1957.
58) Watson, M. L. and Siekevitz, P.: Cytochemical studies of mitochondria. J. Biophysic. Biochem. Cytol., **2**, 639, 653, 1956.
59) Weiss, L.: A study of the structure of splenic sinuses in man and in the albino rat with the light microscope and the electron microscope. J. Biophysic. Biochem. Cytol., **3**, 599, 1957.
60) Yamamoto, T.: On the relationship between mitochondria and fat droplet in the hepatic cells of mouse after administration of hydrocortisone. Arch. Hist. Jap., **15**, 625, 1958.

EXPLANATION OF PLATES

BC: capillary bile duct
C : chylomicron
CF: collagen fiber
CM: cell membrane
DS: DISSÉ's space
EM: encircling membrane
ER: endoplasmic reticulum
F : fat droplet
FR: ferritin granule
G : granule
INF: infolding of cell membrane
LC: liver cell
LP: lipid particle
LV: lymphatic vessel
M : mitochondria
MB: microbody
MM: mitochondrial membrane
MV: microvilli
NC: nucleus
NCL: nucleolus
NM: nuclear membrane
P: pore
PP: pseudopod
RBC: red blood cell
RER: rough surfaced endoplasmic reticulum
SE: sinusoidal endothelium
SEC: sinusoidal endothelial cell
SER: smooth surfaced endoplasmic reticulum
SL: sinusoidal lumen
SMC: smooth muscle cell
V : vesicle

Plate 1: The relationship between the hepatic sinusoidal endothelial cell and the sinusoidal endothelium is shown. The endothelial cell protrudes into the sinusoidal lumen, and the ends of its cytoplasm are stretched thin to form the sinusoidal endothelium. The microvilli of hepatic cells adjoin the endothelium, and the space between them is optic microscopically called DISSÉ's space. The endothelium is penetrated in places by pores, and shows a meshwork like appearance. The endothelial cell shown in this plate is considered to be of immature type, for its cytoplasm is small, compared with the nucleus.

Plate 2: The endothelial cell of medium maturity. The cell is irregular in shape with many protrusions and infoldings. The GOLGI complex is noted in the nuclear depression.

Plate 3: Mitochondria of sinusoidal endothelial and hepatic cells are shown. Mitochondria of the endothelial cell are small in comparison with those of the hepatic cell, being less than two-thirds the size of the latter. In the endothelial cell rough surfaced endoplasmic reticula with PALADE's RNA granules are noted, but they are far smaller than those in the hepatic cell.

Plate 4: A mature hepatic sinusoidal endothelial cell. The nucleus looks smaller than it realy is, as part of it is outside the plate, but the cytoplasm bulks large. The cell is rich in protrusions and infoldings. Rough surfaced endoplasmic reticula are numerous in

the central part of the cell, i. e., around the nucleus, but smooth surfaced ones are mainly noted in the periphery. Many vacuoles which seem to have resulted from infolding of the cell membrane are noted. Mildly osmiophilic, and irregularly-shaped granules are also noted, but they do not appear to be fat droplets.

Plate 5: Part of a hepatic cell in the fasting state. It is considered that mitochondria and endoplasmic reticula are prone to arrange themselves in a sort of definite direction. Cross section of them are chiefly presented in this plate. Fat droplets are few in the fasting hepatic cdll. Two microbodies are noted in the upper and lower part of the plate, but they are to be discriminated from fat droplets.

Plate 6: A specimen taken immediately after the injection of fat emulsion has been finished. Many fat globules are seen sticking to the surface of the endothelial cell, and some of them are also noted in the cell.

Plate 7: The sinusoidal endothelial cell 5 minutes after injection. Many fat globules are noted in the cell. Several globules are taken into the cell as a unit. In the left and right sides of the plate two groups of fat globules are seen encircled by the encircling membranes. In the lower left an infolding is noted which seems to have formed to catch fat globules.

Plate 8: The sinusoidal endothelial cell 10 minutes after injection. Fat globules have thronged into the cell, and forming units, have been placed in vacuoles. Some of these globules are noted to have coalesced with one another into larger droplets in vacuoles.

Plate 9: The sinusoidal endothelial cell 5 minutes after injection. Many fat globules are seen on the surface of the cell. In the cell is noted one large fat droplet which has been built up through coalescence of several fat globules. Just above this droplet fat globles are seen running in a row; it is considered that they are being taken one in succession from the cell membrane into the cell.

Plate 10: The hepatic sinusoidal endothelial cell one hour after injection. The encircling membrane is still noted in a half-disintegrated state around a coalesced fat droplet. Many mitochondria are piled around the fat droplet. From the upper to the middle of the right side of the plate many particles of fat 300～600Å in diameter are seen present in Disse's space.

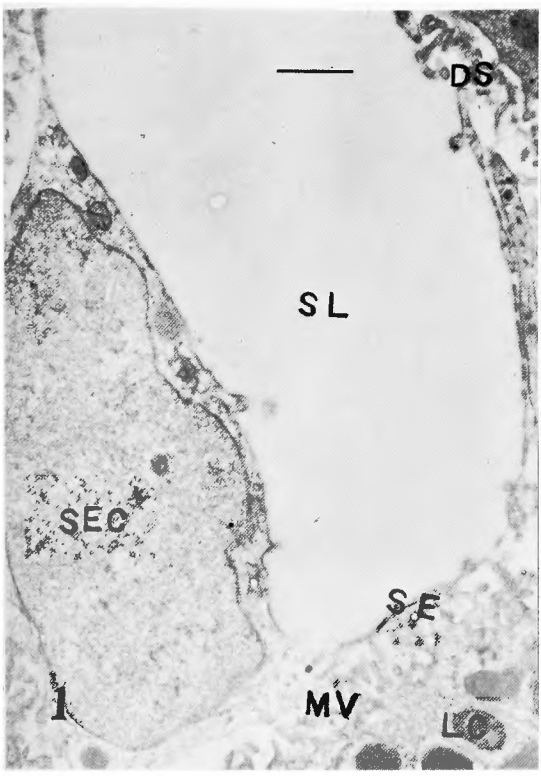
Plate 11: Particles of fat 300～600Å in diameter in Disse's space. These particles are considered to be in transit through the blood stream to hepatic parenchymal cells after having undergone the primary metabolic process in reticuloendothelial cells. But from this plate alone it is impossible to identify them as particles of lipoprotein.

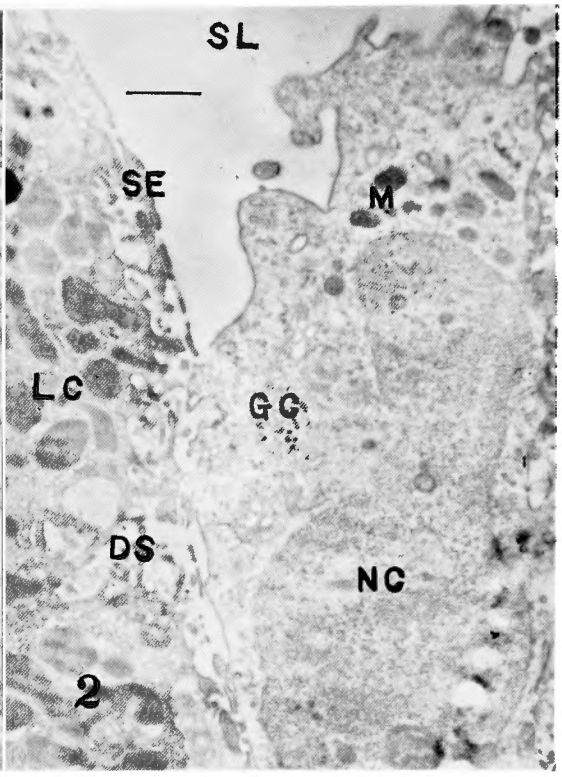
Plate 12 and 13: Hepatic sinusoidal endothelial cells one hour and a half after injection. Some vacuoles are still seen with fat droplets in themselves, but with other vacuoles the encircling membranes have disappeared, and the droplets previously contained in them are discharged into the cytoplasm. These discharged droplets are in contact with mitochondria. Especially in Plate 13 cristae mitochondriales are placed at right angles to droplets, and the part of the outer membrane of the mitochondrium touching the droplet has become indistinct. The droplet itself is observed to have become irregular in shape.

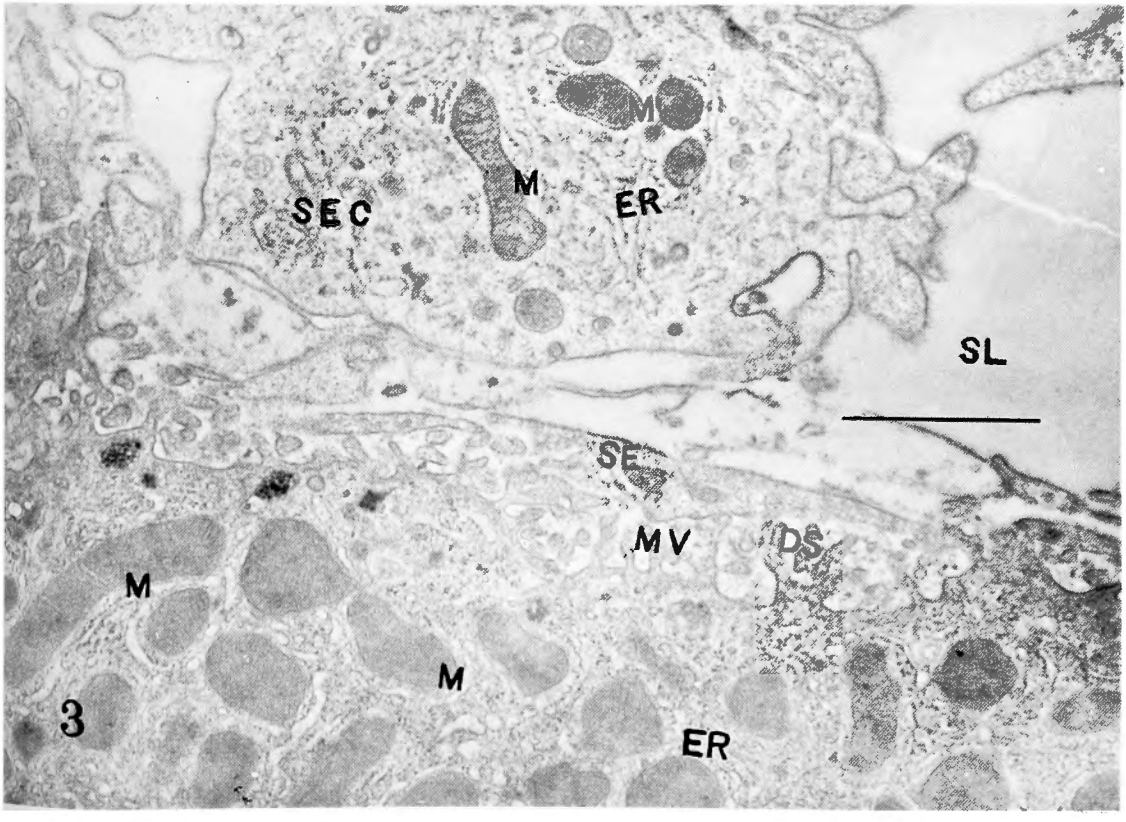
Plate 14: The hepatic sinusoidal endothelial cell two hours after injection. The fat droplet has further become irregular in shape. Mitochondria in contact with it are also observable.

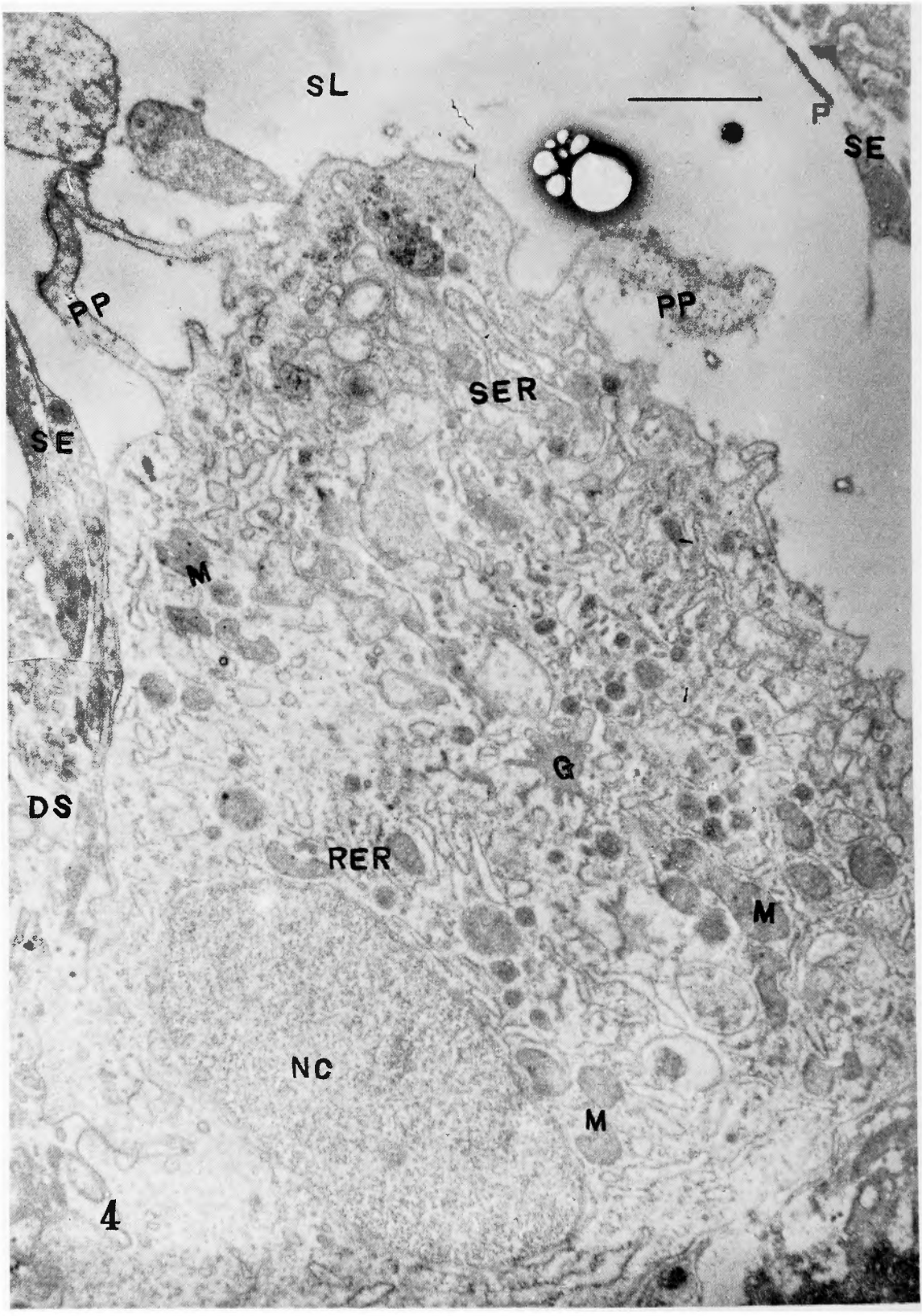
Plate 15: Three hours after injection. The fat droplet has become more distored, and smaller. In the lower right side of the plate is an erythrocyte phagocytized. Split into granular particles, it is undergoing intracellular metabolism.

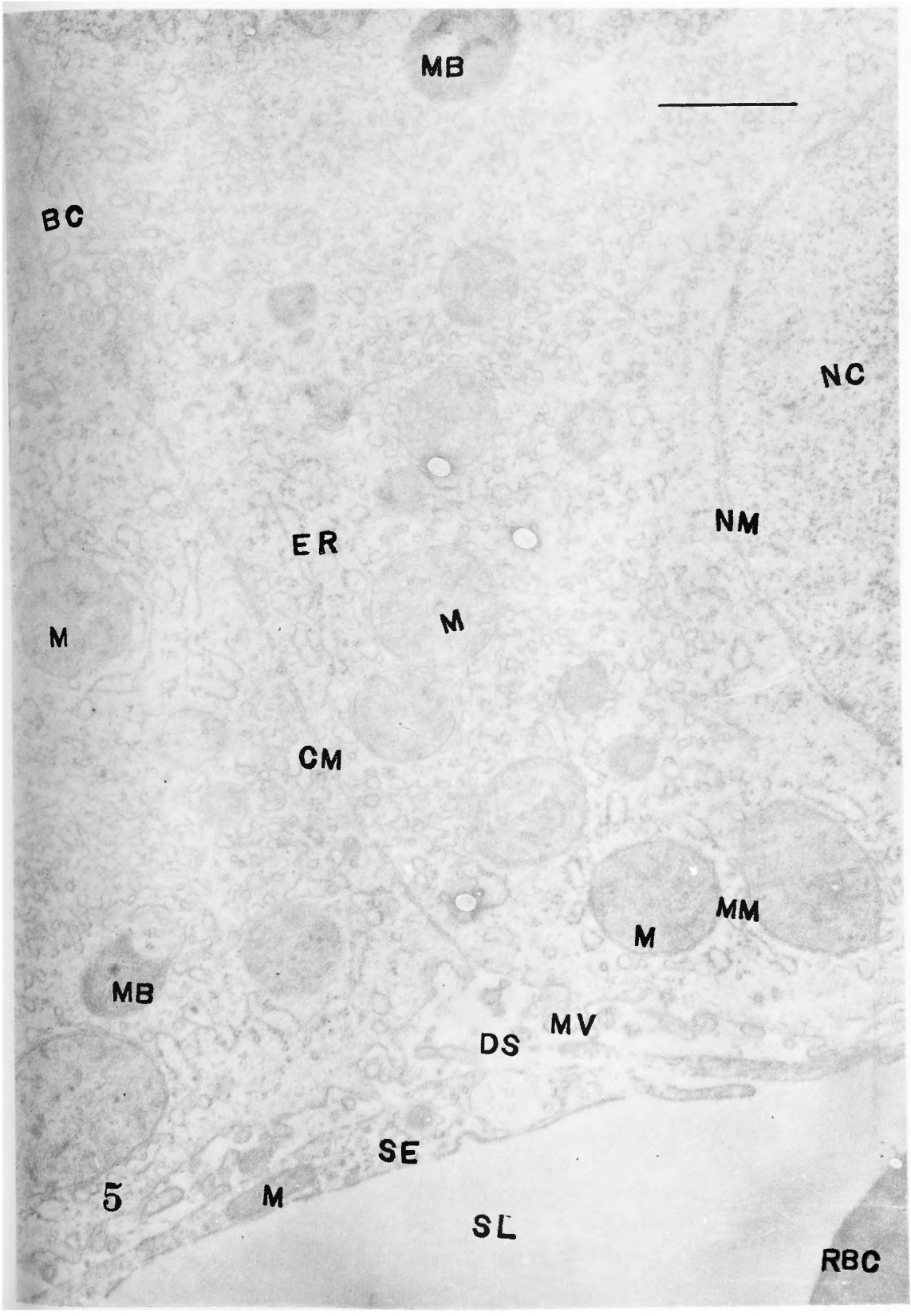
Plate 16: The hepatic parenchymal cell in a section prepared three hours after injection of a 20% fat emulsion in 1.0g of fat per kg body weight quantities. Many fat droplets are observable in the cell, but they have not directly come into the cell from the injected emulsion. It is considered that fat droplets in the emulsion first undergo the primary metabolic process in reticuloendothelial cells; and that the metabolites thus produced are then transferred into hepatic parenchymal cells to be formed

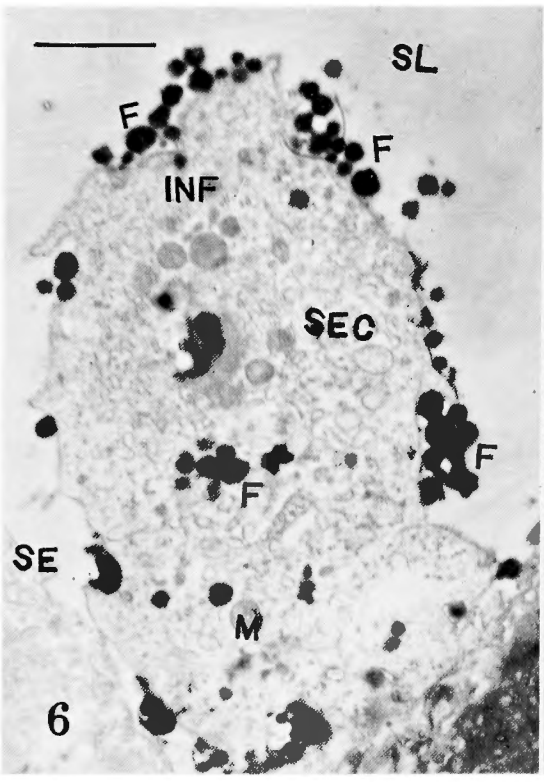

DS
SL
SEC
SE
1
MV
LC

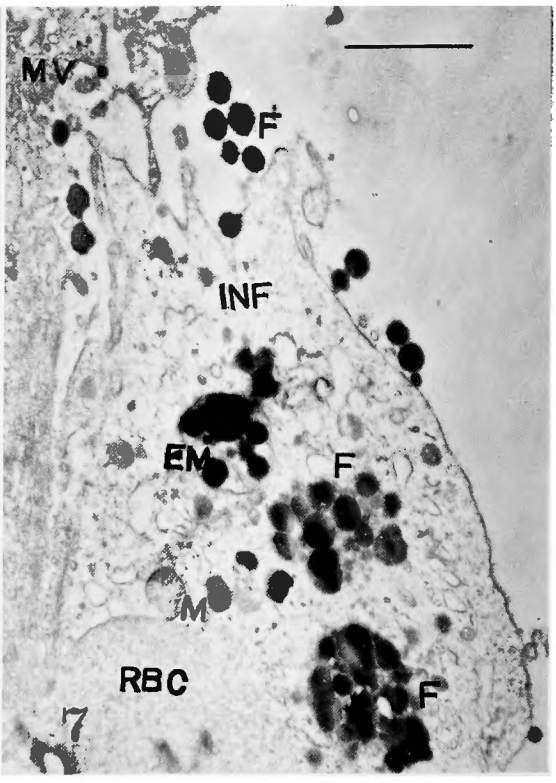

SL
SE
M
LC
GG
DS
NC
2

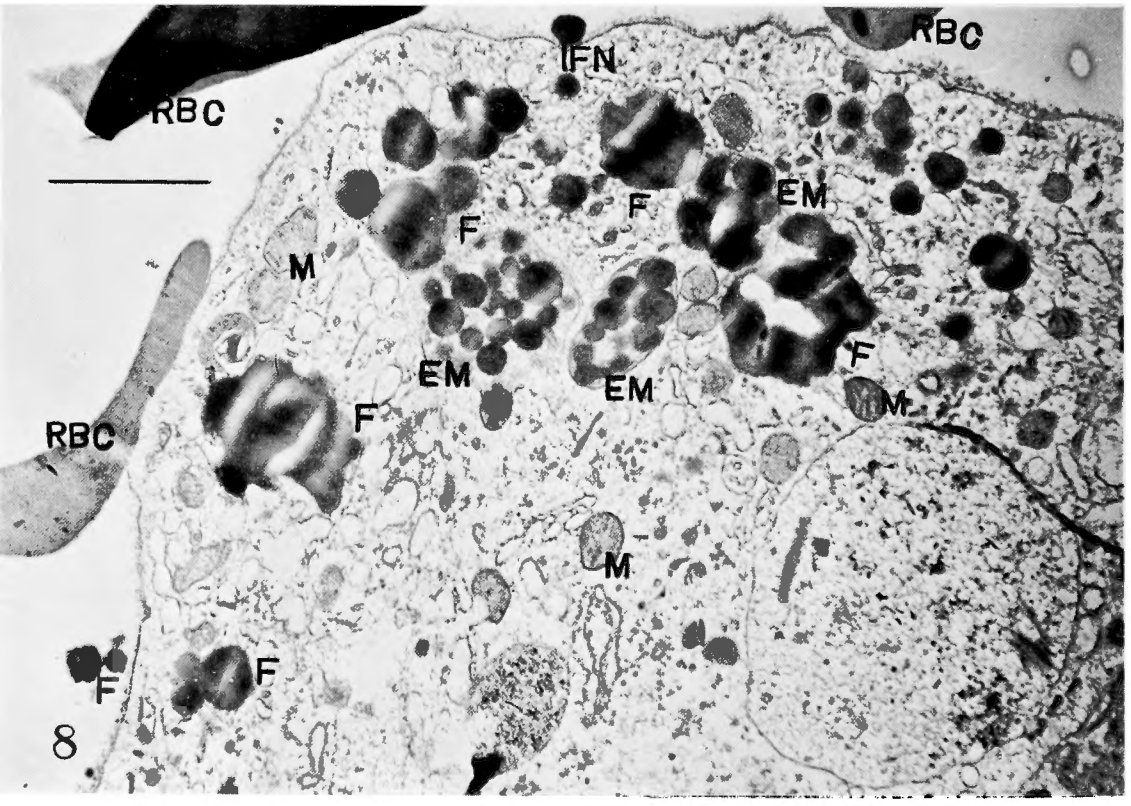

M
M
SEC
ER
SL
SE
MV
DS
M
M
3
ER

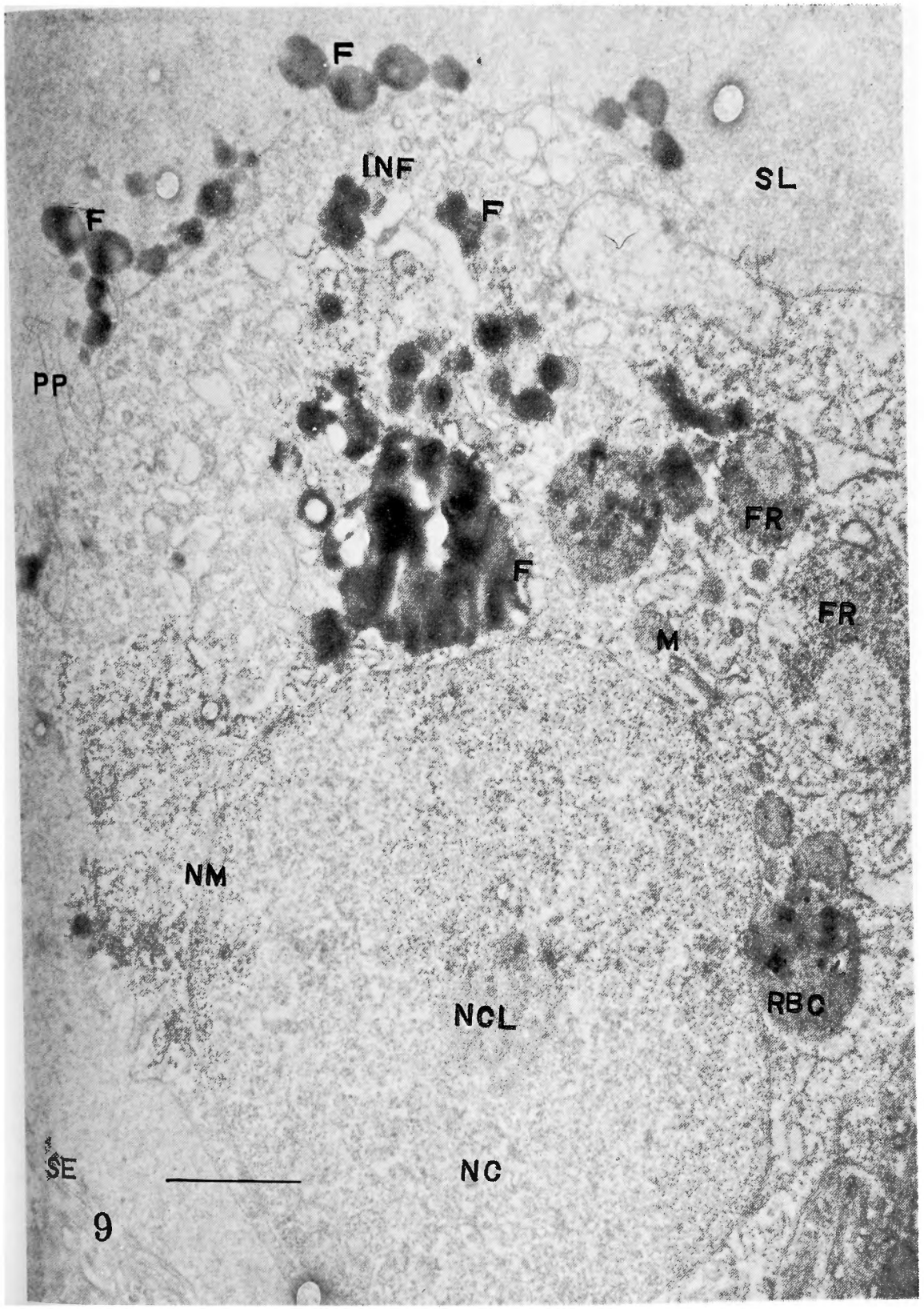

SL
P
SE
PP
PP
SER
SE
M
G
DS
RER
M
NC
M
4


MB
BC
NC
NM
ER
M
M
CM
MM
M
MB
MV
DS
SE
5
M
SL
RBC

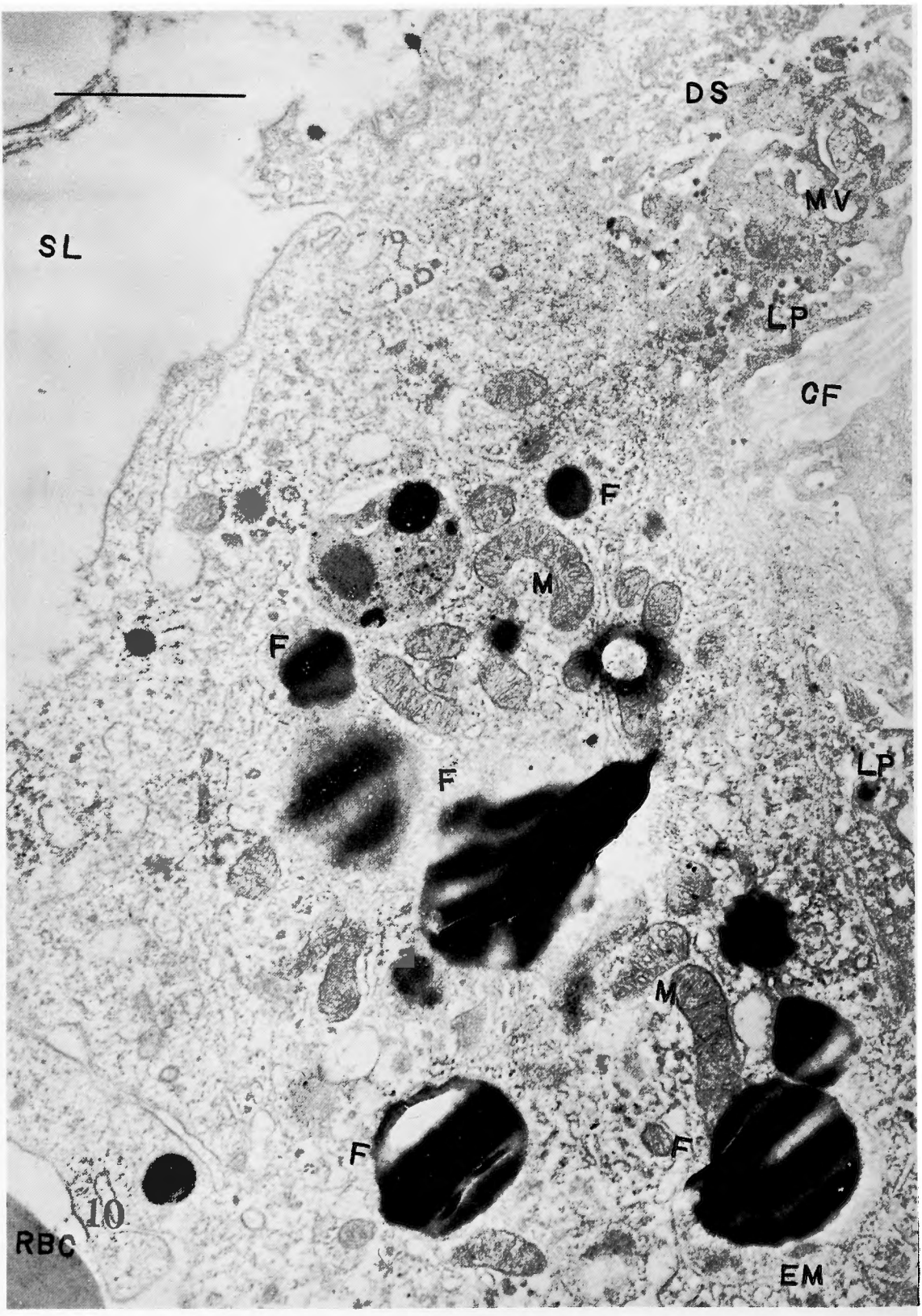

SL
F
F
INF
SEC
F
F
SE
M
6

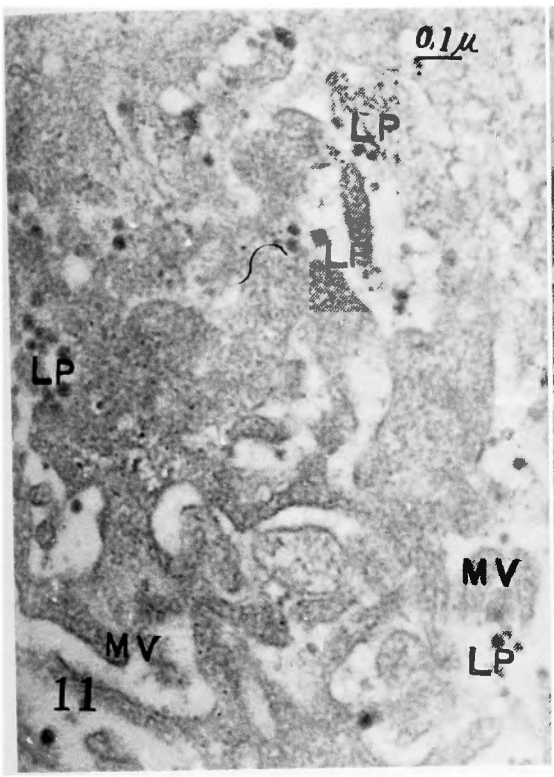

MV
F
INF
EM
F
M
RBC
F
7

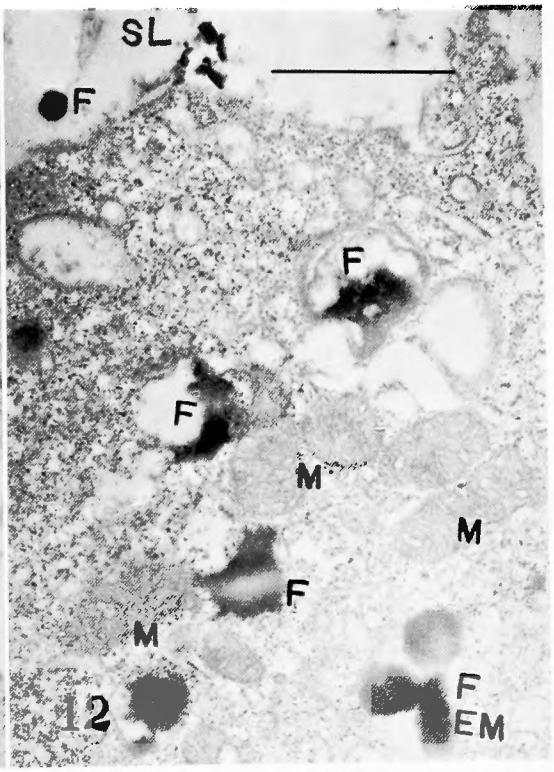

RBC
IFN
RBC
F
F
EM
M
F
EM
EM
F
M
RBC
M
F
F
8

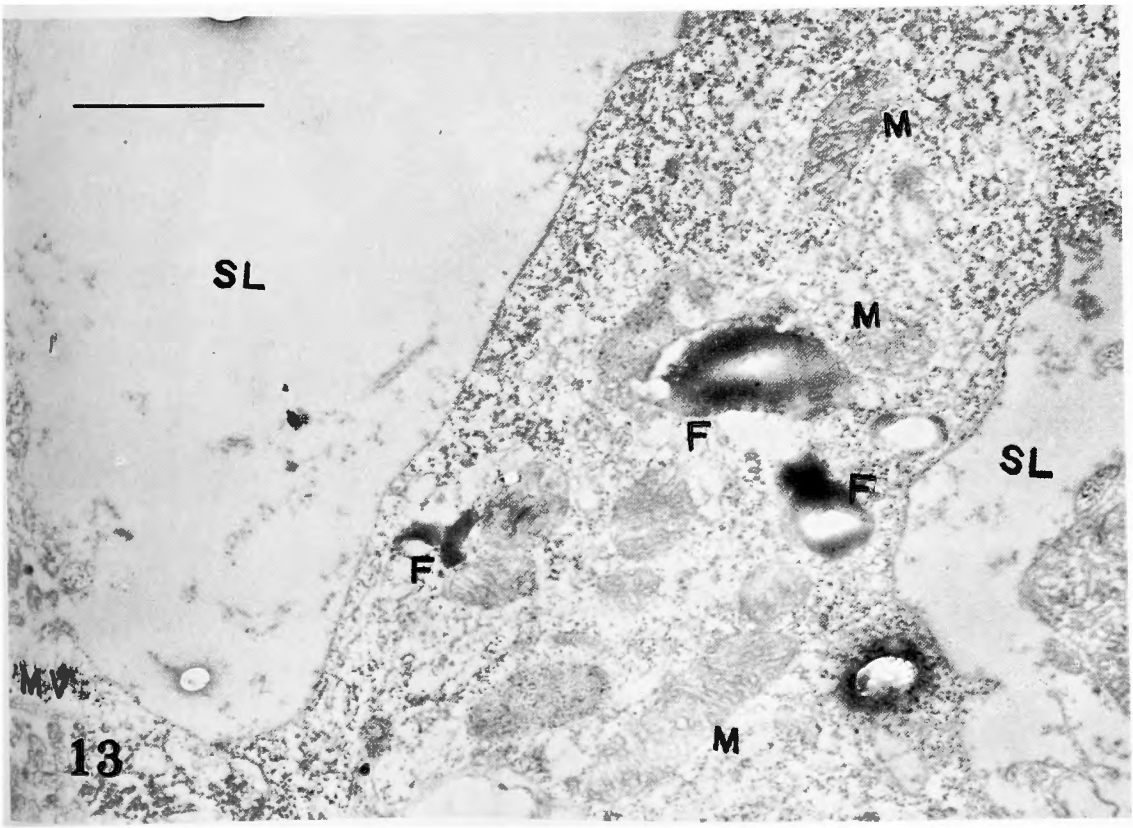


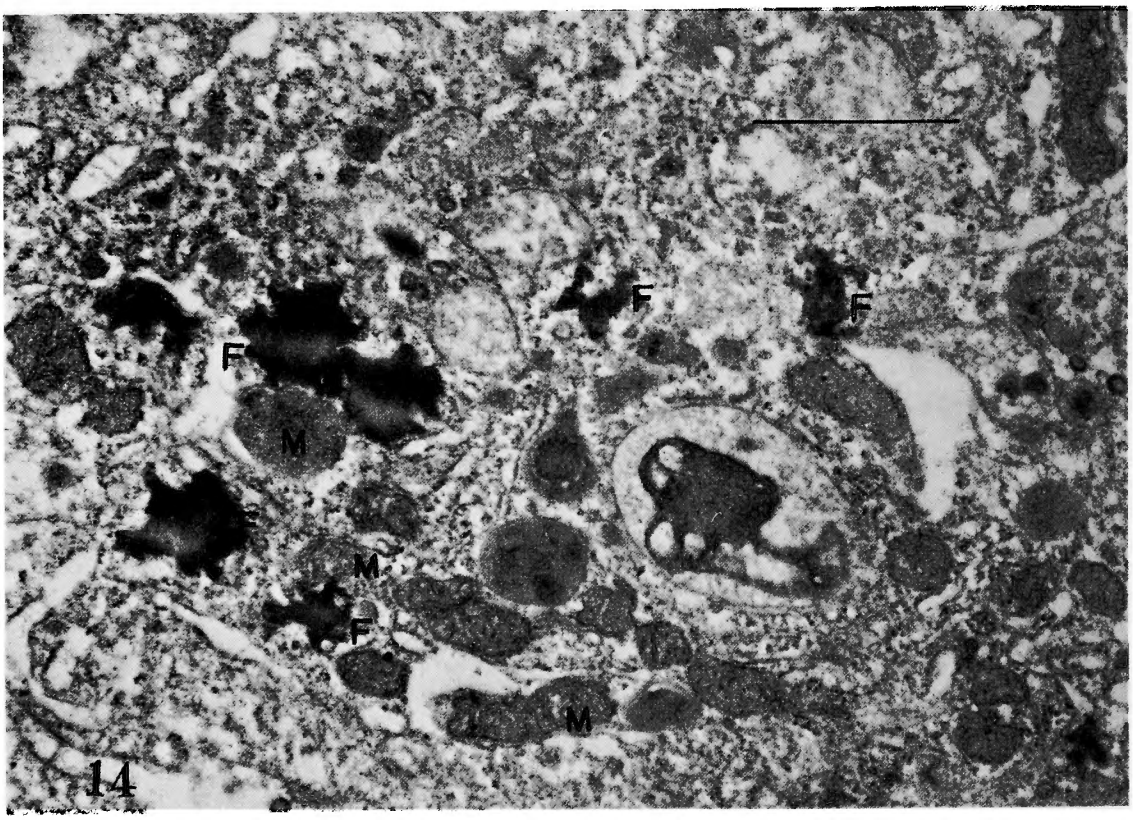
9

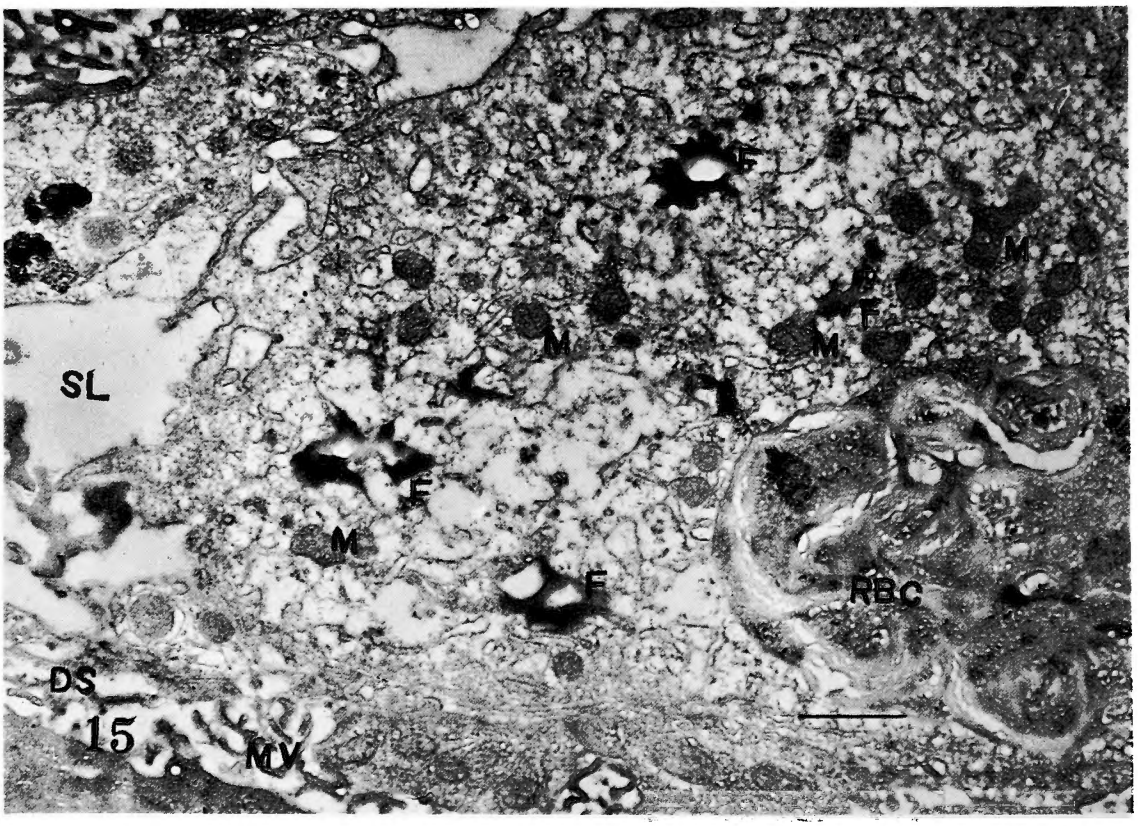

DS
MV
SL
LP
CF
F
M
F
F
LP
M
F
F
10
RBC
EM

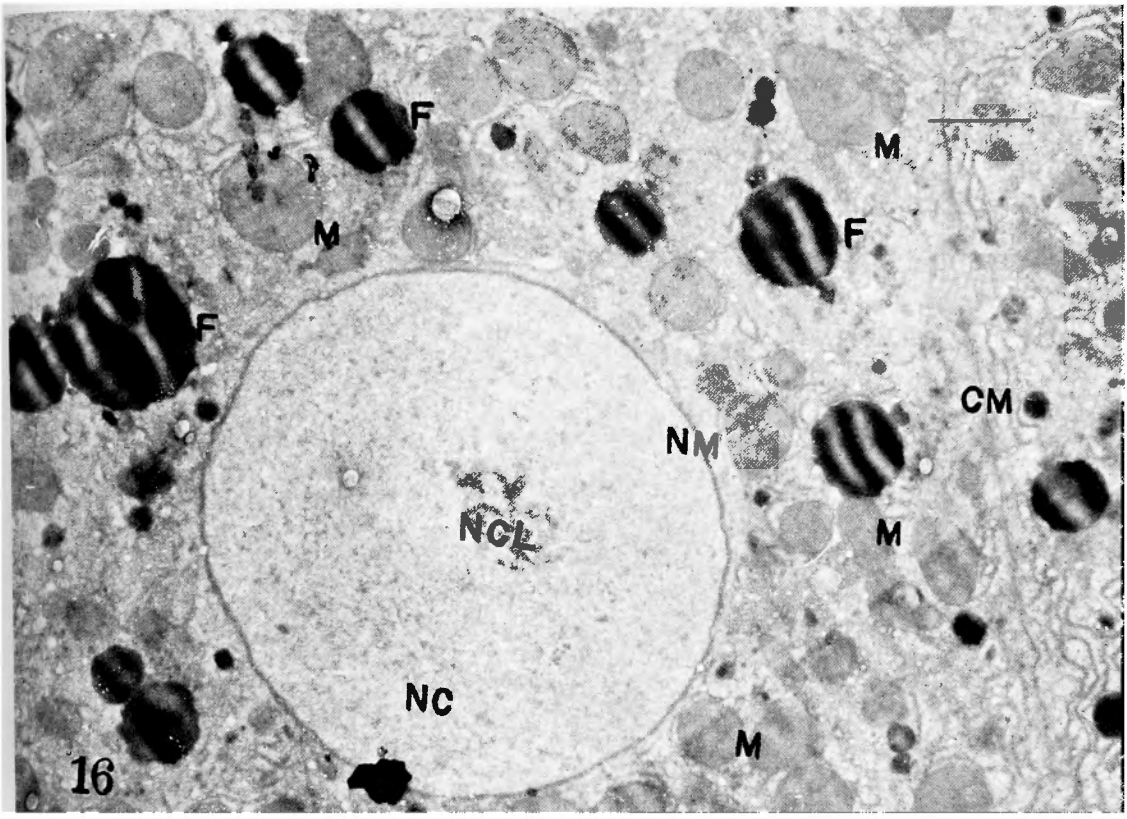

0.1μ
LP
LP
LP
MV
MV
LP
11

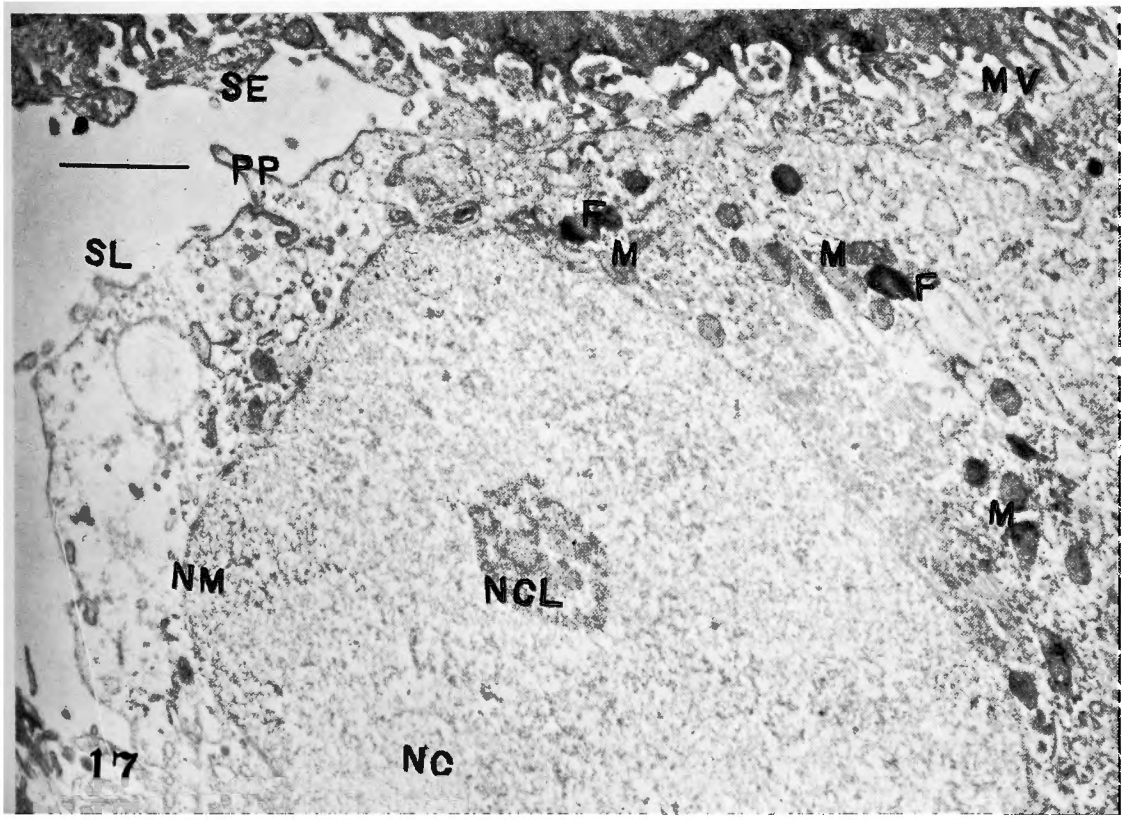

SL
F
F
F
M
M
F
M
F
EM
12

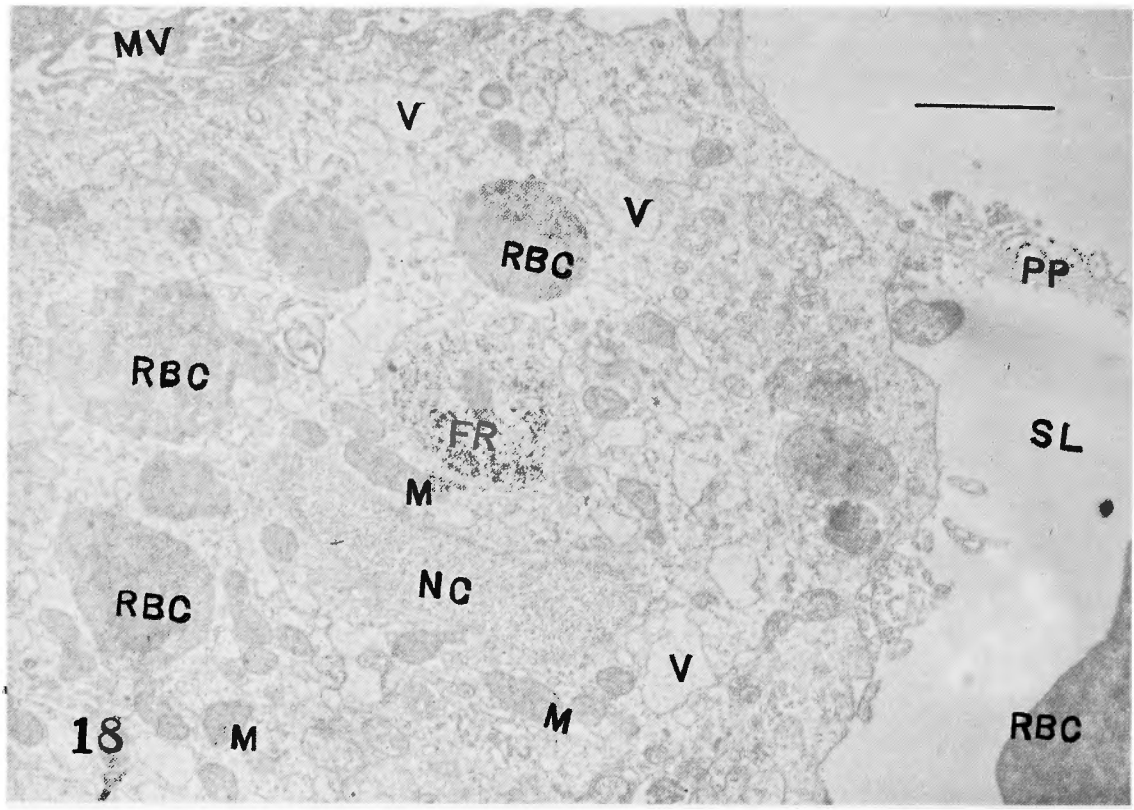

M
SL
M
F
F
SL
F
F
MV
M
13

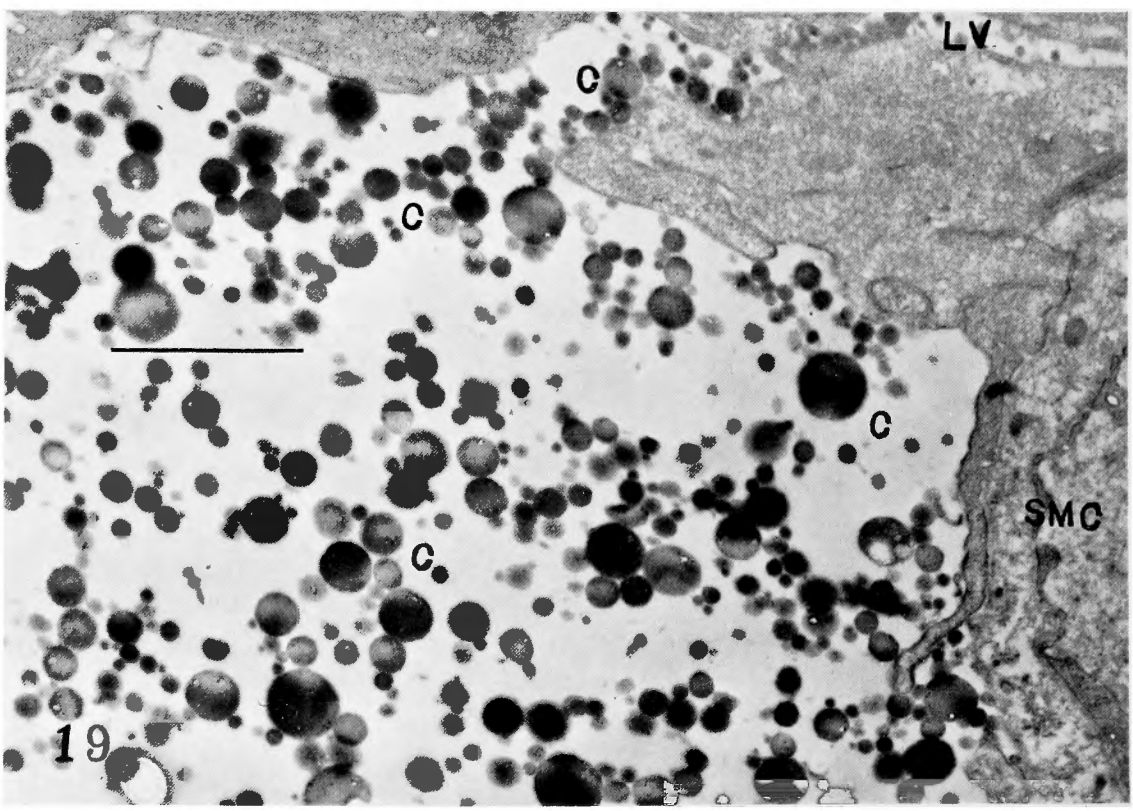

F
F
F
M
M
F
M
14


F
M
M
F
M
SL
F
M
F
RBC
DS
15
MV


F
M
F
M
F
CM
NM
NCL
M
NC
M
16


SE
MV
PP
SL
F
M
M
P
M
NM
NCL
17
NC


MV
V
V
RBC
PP
RBC
FR
SL
M
NC
RBC
V
M
RBC
18
M


LV
C
C
C
C
SMC
C
19

again into fat droplets.

Plate 17: The hepatic sinusoidal endothelial cell 4 hours after injection. Fat droplets are few in the cell.

Plate 18: Six hours after injection. No fat dropelets, but some phagocytized erythrocytes are noted. Ferritin granules discharged from some of them are also noted.

Plate 19: Chylomicra in the lymph vessel of the submucous tissue of the small intestines three hours and a half after oral administration of a sesame oil emulsion in 8.0g of fat per kg body weight quantities. They are all less than 0.5μ in diameter, but differ noticeably in size. In contrast to them, globules of our fat emulsion which are shown in Plates 7, 8 and 9, are less than 0.3μ in diameter and uniform in size.

和文抄録

経静脈性に投与した脂質乳剤の肝臓に於ける処理態度についての電子顕微鏡学的研究

京都大学医学部外科学教室第2講座（指導：青柳安誠教授）

中　村　正　則

われわれの教室では外科的手術前後の非経口的栄養補給の目的で，静脈内へ安全に注入し得る脂質乳剤の製剤化を企図し，それに成功すると共に更にそれを応用して生体内に於ける脂質の中間代謝過程の究明に努力して来た．その結果，われわれは静脈内へ注入された脂質の微粒子はまず全身の網内系細胞群によつて摂取され，それら細胞内で1次的処理をうけたのち，Phospholipid の型で流血を介して全身の実質臓器内へ移行し，そこで初めて2次的処理を受けることを光学顕微鏡学的に略々明らかにすることが出来たのである．

本研究では，このような光学顕微鏡学的に得られた脂質微粒子の生体に於ける処理過程についての見解が果して妥当なものであるか否かを肝臓を対象として，高度の分解能をもつ電子顕微鏡を以て詳細に検討して，特に網内系細胞内に摂取された脂質の処理される過程を形態学的に追究した．即ち，a) 血中へ流入した Glyceride の微粒子はどのような機序で網内系細胞に摂取されるか，b) 次いでそれら細胞内に摂取されたGlycerideはそれら細胞内でどのような変化をうけるか，c) 更にその際，それら細胞内に存在する小器官がどのような態度を示すか，ということを電子顕微鏡学的に匡したのである．そして次の結論に到達した．

1) 脈管内へ注入された Glyceride の微粒子は既に注入5分後で肝静脈洞内皮細胞（Sinusoidal endothelium）によつて多数摂取されるが，時間の経過と共にその程度は益々顕著となる．而もこの際，静脈洞内に認められる Glyceride の微粒子は何れも直径0.3μ以下の大きさで，経口的に脂質を投与した際に小腸の粘膜下組織中のリンパ管内に吸収された Chylomicron の中の最も大きいものは直径 0.5μ である．

2) 肝静脈洞内皮細胞群によつて摂取された Glycerideの微粒子ははじめ洞内皮細胞の細胞膜に由来する Encircling membrane によつて1個乃至数個宛が1単位として摂取され，また数個のものが一括摂取された際にはその Glycerideの微粒子はやがてこれらの貪喰胞中で互いに癒合する．次いでこの Encircling membrane は消失し，結局摂取された脂質は完全に内皮細胞内に遊離されるようになる．

3 Encircling membrane が不完全な構造を示す頃になると，それら内皮細胞中の Mitochondria はその数を増し，且つ，脂質滴の周辺に集積し，屢々脂質滴と機能的に接触するが，この際，Mitochondria の Crista は脂質滴に垂直になるような配列様式をとると同時に，脂質滴と接触した部分の Mitochondria の外膜も不明瞭となる．斯くして Mitochondria の作用下に，脂質滴の形状は不規則且つ不鮮明となつて，やがて静脈洞内皮細胞内から完全に消失する．

4) その頃になると，所謂肝臓の Disse 氏腔にα-

Lipoproteinの形成に与る脂質（その大部分がPhospholipidであるとされている）粒子の大きさに相当する300Å前後の脂質の微粒子が多数出現し，同時に肝実質細胞内へそれは次第に移行する．

5) 要するに以上の所見は，教室の先人がさきに組織顕微化学的並びに生化学的に見出した脈管内注入Glycerideの処理過程についての見解の妥当性を更に電子顕微鏡学的に明白に裏付け得たもので，即ち，脈管内へ流入したGlycerideは肝臓に於てはまずその静脈洞内皮細胞によつて摂取され，それら細胞内でGlycerideはMitochondriaの作用下にPhospholipidに変化した後，α-Lipoproteinの型で流血を介して肝実質細胞内へ移行し，その後の処理過程を受けていることを明白化しえたのである．